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Use of Rubella Virus E1 Fusion Proteins for Detection of Rubella Virus Antibodies

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Two glutathione *S*-transferase fusion proteins containing 44 (p1503) and 75 (p1509) amino acid residues of the rubella virus E1 glycoprotein were expressed in *Escherichia coli* with the aim of producing a recombinant rubella virus antigen for use in serological assays. p1503 contained three neutralizing and hemagglutinating epitopes (G. M. Terry, L. M. Ho-Terry, P. Londesborough, and K. R. Rees, Arch. Virol. 98:189–197, 1988); p1509 contained the putative neutralization domain described by Mitchell et al. (L. A. Mitchell, T. Zhang, M. Ho, D. Decarie, A. Tingle, M. Zrein, and M. Lacroix, J. Clin. Microbiol. 30:1841–1847, 1992) in addition to the three epitopes present in p1503. Both fusion proteins were soluble and affinity purified on glutathione-Sepharose 4B. In Western blots (immunoblots), p1503 and p1509 reacted with human sera containing rubella virus-specific immunoglobulin G. When used as antigens in indirect enzyme immunoassays to detect rubella virus-specific immunoglobulin G, p1503 correctly identified the rubella virus antibody status of 43 (76.8%) and p1509 correctly identified that of 48 (85.7%) of 56 serum samples received for routine rubella virus antibody screening. The results obtained with p1509 compare well with those obtained with a latex agglutination assay.

Rubella (German measles) is a mild, self-limiting disease, but if acquired in the first 12 weeks of pregnancy, it will usually cause congenital infection and abnormalities (2). The aim of rubella vaccination programs is to eradicate congenital rubella by immunizing susceptible women of child-bearing age as well as children of both sexes. Susceptible women are identified by rubella virus (RV) antibody screening programs, and it is current practice in the United Kingdom to screen all women attending antenatal clinics (approximately 650,000 [7]). Screening is also offered by general practitioners, family planning clinics, and occupational health departments (1). Other countries also offer RV antibody screening to adult women; thus, a large number of RV antibody screening tests are conducted each year.

RV is a non-arthropod-borne togavirus and the sole member of the genus *Rubiviridae*. The RV particle is composed of three structural proteins, the capsid protein, C (33 kDa), and two envelope associated glycoproteins, E1 (58 kDa) and E2 (42 to 47 kDa) (17, 23). The E1 glycoprotein appears to be immunodominant (5, 6), and several hemagglutinating and neutralizing epitopes have been identified (15, 21, 24, 25). A neutralizing epitope has also been identified on the E2 glycoprotein (9). No major antigenic variation among strains of RV isolated between 1964 and 1987 in Europe, America, and Japan was identified, suggesting that E1 epitopes are highly conserved (4).

The RV antigens currently used in serological assays are prepared by concentrating virus particles from the maintenance medium of infected cell cultures (3). This type of antigen preparation exhibits considerable batch-to-batch variation, and careful standardization is therefore required prior to use. Such antigens are also relatively expensive to produce and potentially infectious. A recombinant antigen produced in *Escherichia coli* would be considerably easier to produce and standardize. In previous attempts to produce RV E1 fusion proteins in *E. coli*, protein A (22) and an undefined 2-kDa sequence derived from vector LB03 (13) have been used as fusion partners. Protein A fusions required rigorous blocking of nonspecific antibody binding when used in enzyme immunoassays (EIAs) (because of high affinity binding of immunoglobulin G [IgG] by protein A). The use of vector LB03 resulted in the production of a predominantly insoluble product localized in inclusion bodies which required extraction in 8 M urea before small amounts of usable E1 fusion protein were obtained.

We have previously expressed the entire RV E1 glycoprotein coding sequence and a number of subfragments in *E. coli*, using glutathione *S*-transferase (GST) (20) as a fusion partner. GST fusion proteins containing the entire E1 coding sequence and larger subfragments were expressed in forms which could not be purified by affinity chromatography with immobilized glutathione and were therefore presumed to be expressed within insoluble inclusion bodies. However, GST fusion proteins containing 44 to 75 amino acid residues of the E1 glycoprotein were soluble (16). Here we report the use of these soluble GST-E1 fusion proteins in enzyme immunoassays (EIAs) for RV-specific IgG antibodies.

MATERIALS AND METHODS

Generation of RV E1 sequences for cloning and expression. cDNA fragments corresponding to E1 sequences 8977 to 9111 (to produce pUS1503) and 8884 to 9111 (to produce pUS1509) were amplified by PCR, using the primers shown in Fig. 1 and an E1 cDNA as template (16). PCR amplifications were performed in a 50-μl reaction volume containing 1 mM (each) primer, 1 ng of template DNA, 1.5 mM $MgCl_2$, 2.5 U of *Taq* polymerase, 200 μM (each) dATP, dCTP, dGTP, and dTTP, and supplied reagent buffer. Following denaturation for 5 min at 95°C, amplification was performed, using 25 cycles of annealing (50°C, 1 min), extension (72°C, 1 min), and denaturation (95°C, 1 min). Synthesis was completed by a final extension for 7 min at 72°C. Amplified DNA fragments for cloning were purified by gel electrophoresis followed by binding and elution from

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NA45 paper to produce pUS1503 or by the use of PCR cleanup columns (Promega, Southampton, United Kingdom [U.K.]) to produce pUS1509.

Purified fragments were cloned into *Eco*RI- or *Bam*HI-*Eco*RI-digested, dephosphorylated (calf intestinal phosphatase; Boehringer Mannheim, Lewes, U.K.) pGEX-2T (Pharmacia, St. Albans, U.K.), and ligation reactions were transformed into *E. coli* DH5α (Life Technologies, Renfrewshire, U.K.) according to the manufacturer's instructions.

Expression of GST-E1 fusion proteins. *E. coli* DH5α containing pUS1503, pUS1509, or pGEX-2T (as a negative control) was inoculated into 50 ml of L broth containing 100 μg of ampicillin per ml, and the mixture was incubated at 37°C for 18 h. Cultures were then diluted 1:10 with fresh L broth containing 100 μg of ampicillin per ml and incubated for a further 2 h at 37°C. Cultures were induced with 1 mM IPTG (isopropyl-β-D-thiogalactopyranoside; Sigma, Poole, U.K.) and harvested after 3 h of incubation at 37°C.

Purification of GST-E1 fusion proteins. After harvesting, cultures were centrifuged at 8,000 × *g* for 10 min, and the resulting pellet was resuspended in 10 ml of lysis buffer (50 mM Tris-HCl [pH 8.0], 1 mM EDTA, 100 mM NaCl). Cells were lysed by the addition of 0.8 ml of lysozyme solution (10 mg of lysozyme per ml derived from chicken egg white [Sigma] in lysis buffer) and 20 min of incubation at 4°C. Sodium deoxycholate, 40 mg, was added, and the mixture was incubated at 37°C for 20 min. DNase I (200 μl; from bovine pancreas [Boehringer Mannheim]; stock solution [1 mg/ml] in lysis buffer) and 308 μl of 1 M $MgCl_2$ were then added, before incubation at room temperature, until the lysate viscosity disappeared (approximately 20 min). Triton X-100 was added to a concentration of 1%, and cell debris was pelleted by centrifugation at 9,500 × *g* for 5 min. One milliliter of a 50% slurry of glutathione-Sepharose 4B (Pharmacia) prepared according to the manufacturer's instructions was then added to the supernatant, and the mixture was gently stirred for 5 min. Beads were pelleted by centrifugation at 200 × *g* for 5 min and washed three times in ice-cold phosphate-buffered saline (PBS), and bound fusion proteins were then removed by two sequential elutions with 1.0 ml of 50 mM Tris-HCl (pH 8.0) containing 10 mM reduced glutathione (Boehringer Mannheim). Eluates were pooled and stored at −20°C.

Immunoblotting studies. Bacterial lysates containing GST-E1 fusion proteins and GST (as control) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotted (immunoblotted) onto Hybond C super nitrocellulose (Amersham, Little Chalfont, U.K.), using a semi-dry blotter or Transblot apparatus (Bio-Rad, Hemel Hempstead, U.K.). Transfer of proteins from acrylamide gels to the nitrocellulose membrane was confirmed by staining with ponceau-S (Sigma). Nonspecific binding was blocked with 5% skim milk powder in TBST (20 mM Tris-HCl [pH 7.6], 137 mM NaCl, 0.1% Tween 20) overnight at room temperature. GST-E1 fusion proteins were detected with a pool of two RV-positive human serum samples which was preadsorbed with disrupted *E. coli* DH5α containing pGEX-2T without an insert for 3 h at 37°C and then overnight at 4°C. Pooled sera were diluted 1:1,000 in TBST containing 3% bovine serum albumin (BSA), incubated with the blot for 1 h at room temperature, and washed three times with TBST. Visualization of proteins was by incubation with rabbit anti-human IgG Fab_2-horseradish peroxidase (Dako, High Wycombe, U.K.) at 1:5,000 in 3% BSA in TBST for 1 h at room temperature; this was followed by three washes with TBST and use of the ECL detection system (Amersham).

Sera. A panel of 56 serum samples was selected from samples received for routine RV antibody screening by the Diagnostic Virology Laboratory at St. Thomas Hospital. Apart from the inclusion of sufficient numbers of RV-specific IgG positive and negative samples for estimation of assay sensitivity and specificity, sera were chosen at random. All sera were tested by latex agglutination (LA) and EIA, using cell culture-derived RV antigen. Those giving weak-positive or negative results in LA were also tested by single radial hemolysis (SRH). A serum sample was defined as positive if RV-specific antibodies were detected by either LA, SRH, or EIA, using cell culture-derived RV antigen.

LA. Sera were screened for RV antibodies by LA (Rubalex; Orion Diagnostics, Espoo, Finland) according to the manufacturer's instructions. Sera that gave partial agglutination were reported as weak-positive.

SRH. Sera were tested by SRH by the method described previously (3).

EIA with cell culture-derived RV antigens to detect RV-specific IgG. RV and control antigens (prepared as described previously [3]) were added to enzyme-linked immunosorbent assay (ELISA) plates (Maxisorp; Nunc, Roskilde, Denmark) at a 1:200 dilution (optimal) in coating buffer (0.02 M Tris, 0.003 M EDTA, 0.3 M NaCl, 34 mM $CaCl_2 \cdot 2H_2O$ 24.5 mM $MgCl_2 \cdot 6H_2O$; pH 7.8), and the plates were incubated at 4°C for 18 h. Plates were washed three times with PBS containing 1% Tween 20 (PBS-T) using an automated plate washer (Welltech; Denley, Billingshurst, U.K.); 400 μl of blocking agent (1% BSA in coating buffer) was then added to each well and the plates were incubated at 37°C for 1 h. Serum samples diluted 1:100 (optimal) in PBS containing 5% inactivated fetal calf serum and 0.05% Tween 20 were added in duplicate to both virus and control antigen-coated wells, and the plates were incubated at 37°C for 1 h. After the plates were washed, peroxidase-conjugated anti-human IgG (Dako) was added at a 1:2,000 dilution (optimal) in serum diluent, the plates were then incubated at 37°C for 1 h. After another wash, 100 μl of substrate solution (0.485 M citric acid, 0.1 M Na_2HPO_4, 3.7 mM *O*-phenylenediamine, 0.4% [vol/vol] H_2O_2) was added to each well and the plates were incubated at 25°C for 7 min in a dark chamber. The reaction was stopped by the addition of 100 μl of 1 M H_2SO_4 per well, and optical densities (ODs) were read at 492 nm with an MR700 plate reader (Dynatech, Billingshurst, U.K.). Delta absorbance values were calculated by subtracting the mean OD value for sample wells coated with cell control antigen from the mean OD value for wells coated with RV antigen. A serum sample was considered positive if its delta OD value was greater than 2 standard deviations above the mean delta OD of a panel of negative sera. Serum background OD values were determined with wells which were not coated with RV antigen but which were otherwise processed as described above.

Use of purified fusion proteins p1503 and p1509 in EIAs to detect RV-specific IgG. Optimal concentrations of sera and reactants were determined in preliminary experiments. Purified fusion proteins were coated onto ELISA plates (Nunc Maxisorp) at a concentration of 5 μg per well in 0.05 M carbonate-bicarbonate coating buffer (pH 9.6; Sigma) at 37°C for 1 h. Plates were washed three times in PBS-T, using an automated plate washer (Denley); nonspecific sites were then blocked by the addition of 1% BSA in coating buffer and by incubation at 37°C for 1 h. Serum samples diluted 1:20 in PBS-T containing 1% BSA (PBS-T-BSA) were added in duplicate to both p1503- or p1509- and GST-coated wells, and the plates were incubated at 37°C for 1 h. After the plates were washed three times as described above, biotin-labelled monoclonal anti-human IgG (Sigma) diluted 1:1,000 in PBS-T-BSA was added and the plates were incubated at 37°C for 1 h. Plates were then washed as described above, extravidin-peroxidase (Sigma) was added at a 1:500 dilution in PBS-T-BSA, and the plates were incubated at 37°C for 1 h. After another three washes, substrate (peroxidase substrate system [Sigma]) was added and the plates were incubated at room temperature in a dark chamber for approximately 10 min. The reaction was stopped by the addition of 25 μl of 3 M H_2SO_4 to each well. ODs were measured at a wavelength of 492 nm, using an MR700 plate reader (Dynatech). Delta absorbance values were calculated by subtracting the mean OD value for sample wells coated with GST alone from the mean OD value for wells coated with GST-E1 fusion proteins. A serum sample was considered positive if its delta OD value was greater than 2 standard deviations above the mean delta OD of a panel of negative sera. Serum background OD values were determined with wells which were not coated with fusion protein but which were otherwise processed as described above.

RESULTS

Production and expression of fusion proteins. cDNAs coding for RV E1 protein sequences between amino acids 243 and 286 and amino acids 212 and 286 were modified for insertion in frame into pGEX-2T to produce, respectively, pUS1503 and pUS1509 (Fig. 1). pUS1503 directs the expression of a fusion protein with a predicted M_r of 30,000, named p1503, while pUS1509 directs the expression of a fusion protein with a predicted M_r of 33,000, named p1509. Production of both fusion proteins was confirmed by SDS-PAGE and Western blotting of lysates from *E. coli* containing pUS1503, pUS1509, or pGEX-2T after induction with 1 mM IPTG (Fig. 2).

Purification of fusion proteins. Fusion proteins p1503 and p1509 and GST (as a control to permit estimation of background OD in EIAs) were affinity purified, using glutathione-Sepharose 4B. The material eluted from the affinity matrix by 10 mM reduced glutathione is shown in Fig. 3. There was some evidence of proteolysis suggested by the presence of lower-M_r species in purified preparations of p1503. Slight contamination with high-M_r proteins was occasionally found in purified preparations of p1503, p1509, and GST.

Comparison of recombinant E1-fusion proteins and cell culture-derived RV antigens in EIAs for the detection of RV-specific IgG. Fifty-six serum samples received for routine RV screening were tested by indirect EIAs, using p1503, p1509, and cell culture-derived RV as antigens. When p1503 was used as antigen, 43 of the 56 serum samples gave results in agreement with cell culture-derived RV antigen. Three serum samples gave false-positive results and 10 serum samples gave false-negative results, giving a sensitivity of 74.3% and a specificity of 82.3% (Table 1 and Fig. 4A). The sensitivity and specificity were improved when p1509 was used as antigen; 48 serum samples gave results in agreement with the cell culture-derived RV antigen, 7 samples gave false-negative results, and 1 serum sample gave a false-positive result (Table 1 and Fig. 4B). One serum sample (serum 8, Table 2) gave a false-positive result with both p1503 and p1509, although no rubella anti-

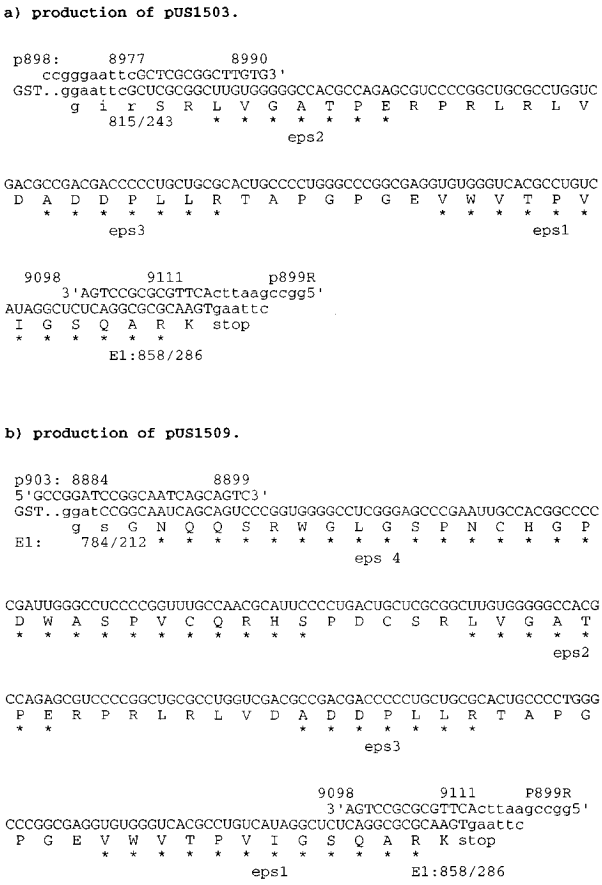

FIG. 1. RV E1 sequences in pUS1503 (a) and pUS1509 (b). Primers used for modification of RV cDNA (p898, p898R, and p903) are shown above the appropriate RV sequence. The reverse primer (p898R) is shown as the antisense sequence. GST nucleotides and amino acids are shown in lowercase letters. RV sequences are in uppercase letters. RV E1 amino acids are numbered according to the E1 sequence (212 to 286) and the RV structural polyprotein sequence (784 to 858). E1 nucleotides are numbered according to their location in the RV genome according to Dominguez et al. (8).

bodies were detected by LA or EIA using cell culture-derived RV antigen. Thus, a sensitivity of 82% and a specificity of 94.1% were obtained with the p1509 antigen. The results obtained with p1509 are in close agreement with those obtained

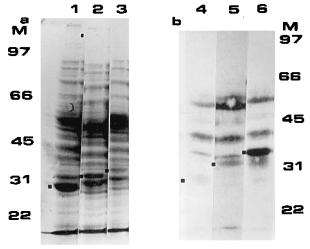

FIG. 2. Production of GST, p1503, and p1509. (a) Coomassie blue-stained SDS-PAGE gel showing lysates of IPTG-induced *E. coli* DH5α producing GST (lane 1), p1503 (lane 2), and p1509 (lane 3). (b) Western blot analysis of IPTG-induced *E. coli* DH5α producing GST (lane 4), p1503 (lane 5), and p1509 (lane 6). The positions of GST and fusion proteins p1503 and p1509 in the appropriate lanes are indicated by the symbol ■. Lanes 1 to 6 were derived from a single gel. The positions of the molecular weight markers (10^3) are shown in the lanes marked M.

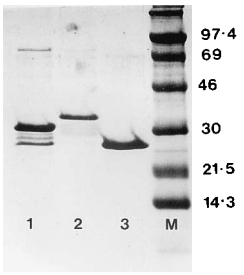

FIG. 3. Purification of GST p1503 and p1509. SDS-PAGE gel showing glutathione-Sepharose 4B affinity-purified p1503 (lane 1), p1509 (lane 2), and GST (lane 3). The numbers indicate the size (10^3) of the molecular weight markers in lane M.

by using LA alone, which correctly identified 51 of the 56 serum samples.

The results obtained with the seven serum samples which gave false-negative results on p1509 are given in Table 2 (sera 1 to 7). Only serum 3 gave a clear positive result in LA; the other six serum samples gave negative or weak-positive results by LA and were therefore referred for further testing. All seven serum samples were positive when tested in an EIA using cell culture-derived antigens.

Reproducibility. The reproducibility of the p1509 antigen EIA was estimated for two serum samples: serum 1, RV-specific IgG positive, mean OD = 0.9578 (standard error of the mean = 0.0142; replicates = 10) serum 2, RV-specific IgG negative, mean OD = 0.064 (standard error of the mean = 0.021; replicates = 9). The background OD for these sera was in the range of −0.013 to +0.033 (mean = 0.0125).

The reproducibility of the cell culture-derived RV antigen EIA was estimated with the assay serum control: mean OD = 0.441 (standard error of the mean = 0.01; replicates = 10). The background OD for this serum on uncoated plates was in the range of −0.004 to +0.023 (mean = 0.006).

DISCUSSION

We have previously reported the expression of the entire RV E1 glycoprotein and truncated E1 proteins in *E. coli* as C-terminal fusions with GST (16). The use of a number of these fusion proteins as antigens in EIAs was compromised by their inability to be purified as soluble proteins by affinity chromatography on immobilized glutathione. Therefore, to determine whether a previously defined epitope-containing region of E1 could be produced as a soluble protein amenable to affinity purification, we expressed a short 44-amino-acid fragment

TABLE 1. Comparison of cell culture-derived RV and recombinant GST-E1 fusion proteins p1503 and p1509 as antigens in EIAs to detect RV-specific IgG[a]

Cell culture-derived RV antigen (no. of samples)	No. of samples of given status with:			
	p1503 antigen		p1509 antigen	
	Positive	Negative	Positive	Negative
Positive 39	29	10	32	7
Negative 17	3	14	1	16

[a] The sensitivity and specificity with p1503 were 74.3 and 82.3%, respectively; those for p1509 were 82 and 94.1%, respectively.

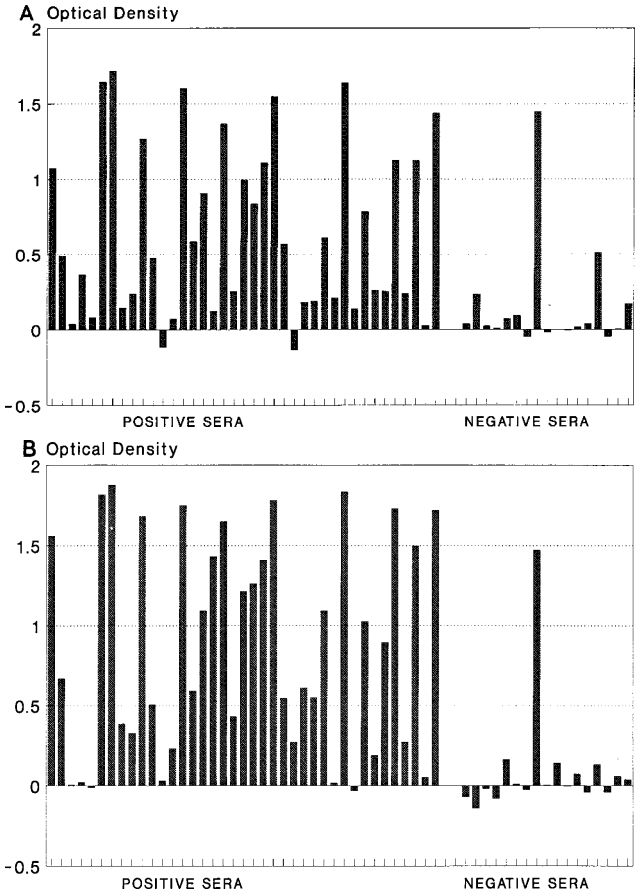

FIG. 4. Comparison of recombinant GST-E1 fusion proteins p1503 (A) and p1509 (B) as antigens in EIAs to detect RV-specific IgG. Abscissa, 56 serum samples received for RV screening; ordinate, delta OD at 492 nm.

(amino acids 243 to 286) of the E1 glycoprotein as a GST fusion. The region between amino acids 243 and 286 contains three epitopes reported by Terry et al. (21) and completely lacks cysteine residues, precluding the formation of aberrant disulfide bonds which could affect fusion protein properties such as solubility. This fusion protein was soluble and could be affinity purified for use in EIAs. We subsequently increased the size of the E1 portion of the fusion protein to 75 amino acids to include the putative neutralization domain identified by Mitchell et al. (15). This fusion protein (p1509), containing E1 residues 212 to 286, was also soluble and could be affinity purified. We noted that as the size of the fusion proteins increased and cysteine residues were incorporated, fusion protein productivity decreased. We cannot say, however, what the main contributory factor to this reduction in productivity is because we have produced larger GST-E1 fusion proteins at higher levels than obtained for p1503 and p1509. These have so far not proved amenable to affinity purification and may well be present as inclusion bodies (unpublished observations).

When used as antigens in EIAs for RV-specific IgG, p1503 and p1509 detected 74.4 and 82.3% of positive sera, respectively. The p1509 results are very encouraging and compare well with those obtained by LA, an assay widely used for RV antibody screenings (19a, 22a). Of the seven serum samples which gave false-negative results, three samples were initially negative by LA and three others gave only weak-positive reactions. Only when tested using cell culture-derived antigen

TABLE 2. Discrepant results obtained when p1509 was used as antigen in EIA to detect RV-specific IgG

Serum no.	Results[a] by:			
	Routine screening assays			EIAs using recombinant protein p1509
	LA	SRH	EIA	
1	ND/WP	ND	D	ND
2	ND/WP	D	D	ND
3	D	D	D	ND
4	WP	ND	D	ND
5	ND	ND	D	ND
6	WP	ND	D	ND
7	WP	D	D	ND
8	ND	ND	ND	D

[a] WP, weak-positive; ND, not detected; D, detected.

EIA were positive results obtained with these six serum samples (Table 2).

The failure of the E1-fusion proteins to detect RV-specific IgG in some sera could be due to differences in the conformation of the E1 moiety of fusion proteins and in mature RV. Studies utilizing murine monoclonal antibodies suggest that the majority of RV neutralizing epitopes are conformational rather than linear (24). The short regions of E1 present in p1503 and p1509 presumably represent linear antigenic domains and may not accurately mimic the three-dimensional structure of mature E1.

False-negative reactions may also occur because some individuals develop antibody responses against regions of E1 not present in p1503 and p1509. We have cloned and expressed regions of the RV E1 glycoprotein which contain neutralizing and hemagglutinating epitopes (15, 21, 24, 25); however, it is possible that some individuals do not produce IgG antibodies against these regions. Mitchell et al. (14), using synthetic E1 peptides in EIA, showed that although E1-213-239 (contained within p1509) was the region of E1 most frequently recognized by positive sera, more than 30% of individuals failed to respond to it. Antibodies to E1-154-179 were detected in approximately 15% of individuals. This region is not present in either p1503 or p1509, and thus it is possible that the incidence of false-negative reactions obtained with p1509 antigen EIA might be reduced by the use of a chimera or mixture of short recombinant RV antigenic domains such that all patterns of antibody responses to E1 antigenic domains would be detectable. Such polyepitope fusion antigens have been used to detect human cytomegalovirus-specific IgG and provide more sensitive assays than recombinant antigens consisting of a single epitope (19).

False-negative reactions could also be due to steric hinderance of the RV component of fusion proteins by the GST fusion partner. Attempts to cleave the GST portion of E1 fusion proteins by using the thrombin site present in GEX-2T-derived fusions were unsuccessful (data not shown). Inefficient cleavage of GST fusion proteins by thrombin possibly arising from masking of the thrombin recognition site by GST has been described previously (18). Others have shown that the introduction of a glycine-rich linker sequence between GST and the thrombin cleavage site increases the efficiency of cleavage (10).

Although *E. coli*-based expression systems such as GST are not capable of authentic glycosylation, it is unlikely that this explains the false-negative results obtained with the p1503 and p1509 antigens. Although glycosylation is required for full immunogenicity, mutant RV E1 glycoproteins deficient in the

three glycosylated side chains found in the mature protein exhibit normal antigenicity (11).

One RV-specific IgG-negative serum sample (serum 8, Table 2) gave false-positive results with p1509, and three serum samples gave false-positive results with p1503. Western blotting studies with serum 8 indicated that the RV sequences present in both p1503 and p1509 were recognized but there was only slight reactivity to GST (data not shown). Thus, it is possible that antibodies to antigens which mimic the structure of the E1 region present in p1509 may be responsible for false-positive reactions. Proteins with sequence homology to RV E1-211-285 include human T-cell surface glycoprotein CD5 precursor, human DNA ligase 1, human protein tyrosine phosphatase delta, human hyanodine receptor, *E. coli* MDL protein, *E. coli* deoxyguanidine triphosphate triphosphohydrolase, *E. coli* transcription antitermination protein NUSG, *E. coli* phosphoribosylformylglycinamidine synthase, *E. coli* catabolite activation-like protein, and *E. coli* hypothetical 26.4-kD protein (Swissprot Database). Putative false-positive reactions have also been described when synthetic peptides corresponding to regions of the hepatitis C virus NS5 protein were used as antigens in PEPSCAN, possibly occurring as a result of a cross-reaction with herpes simplex virus-specific antibodies (10a).

Thus, regions of viral proteins expressed for use as diagnostic antigens should be screened for possible homologies with other antigens which may be encountered by humans. Site-directed mutagenesis of recombinant antigens might be used to limit the degree of homology. Serum antibodies to the contaminating *E. coli* proteins which copurify with p1509 could also give rise to the type of false-positive reaction exhibited by serum 8. False-positive reactions or complications arising from cross-reactivity to *E. coli* proteins have been described when GST fusion proteins were used as antigens for serological diagnosis of lentivirus infection of sheep and goats (26) and bovine viral diarrhea virus infection of cattle (12).

In summary, we have produced two soluble RV GST-E1 fusion proteins and evaluated their use as antigens in EIAs. Initial results with fusion protein p1509 are encouraging; the sensitivity and specificity compared well with a single screening method in current use and might be improved by further purification of fusion proteins. GST fusion proteins are relatively inexpensive to produce, can be purified by a simple one-step procedure, and may be easily standardized. It is possible that the "ideal" recombinant antigen may include a chimera or mixture of epitope-containing regions of RV E1. False-positive reactions may be minimized by careful choice of the regions of RV proteins incorporated into recombinant proteins and site-directed mutagenesis of RV sequences such that cross-reactions are prevented without altering the sensitivity of assays.

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